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The Council of the Agr. Chem. Soc. of Japan has decided to publish English Abstract of those papers appearing in the Journal in a separate form in order to facilitate the circulation in foreign countries.

Bulletin of the Agr. Chem. Soc. of Japan is published for this purpose from May 1926 monthly. The numbering begins with Vol. 2, No. 5. The earlier parts are represented by the English abstracts published in the Journal annexed to the Japanese texts.

The articles to be appeared in the Bulletin must be concise, supplied with experimental methods and data and understandable, without specially referring to the Japanese texts. It ought, however, not exceed four printed pages as a rule. Any longer articles may be accepted according to the decision of the Council, with or without charge for exceeding pages.

Journal of the Agr. Chem. Soc. of Japan will be published in Japanese as formerly. Those desiring the detailed information of the articles appeared in the Bulletin may look for in the Journal of the same Number or the same Volume.

BIOCHEMICAL STUDIES ON BONE, SKIN, HIDE AND SHEAL.

1. SEXUAL DIFFERENCES IN THE COMPOSITION OF BONE. (PRELIMINARY REPORT)

By

HIROMICHI HARA.

(Received 10 th June 1930)

The composition of proteins, fats and others are different by sex. So the bone must also differ in its composition. Not only the contents of phosphorus and calcium, but the structure of bone protein, collagen, and the state of combination with calcium must differ.

On this opinion, the author studied the composition of bone from the standpoint of sex.

Preparation of bones.

The bones were removed from the body immediately after death. The adherent soft tissues were dissected from the bones which were then crushed into small fragments. The crushed bones were immediately extracted in a Soxhlets extraction apparatus with a mixture of ethyl–alcohol and ether for about 5 hours. Before beginning the extraction this mixture was made alkaline with sodium hydroxyde. And extracting temperature not exceeding 38–degrees. The bones were then pulverized finally and passed through an 80 mesh shieve. The preparation of the material for analysis was completed within 24 hours after removed of the specimen from the body.[1]

Determination of total nitrogen and ash.

Total nitrogen was determined by the Kjeldahl method, and the content of ash by the ordinary method.

The results showed that the contents of total nitrogen are higher in the male bone than the female.

Determination of calcium and phosphorus.

10mg. of powdered samples were weighed and transferred to a dry 10c.c. volumetric flask. 2c.c. of 1N HCl added and the mixture was then digested in a boiling waterbath for about 10 minutes. When it was cooled, 3c.c. of 20% trichloroacetic acid were added and the volume was made up to 10c.c. with dist. water. Aftor 30 minutes it was filtered.

2c.c. of the filtrate were used for the determination of calcium and the

other 2c.c. of the filtrate for phosphorus. Calcium was determined by Kramer and Tisdall's method[2] and phosphorus by Bell–Doisy–Briggs' method.[3]

The datas showed that the contents of calcium were superior in femal, and the phosphorus were superior in male.

When these datas were calculated to N=100, the contents of both calcium and phosphorus were superior in femal to male. This shows that the ability of accumulation of nitrogen is inferior in female to male, while the contents of mineral matters in nitrogenous substances superior to male.

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BIOCHEMICAL STUDIES ON *SALMONIDAE*. V.

ON THE INFLUENCE OF THE SUN-LIGHT ON CHEMICAL COMPOSITION OF FISH.

I. ESPECIALLY ON ASSIMILATION OF CALCIUM AND NITROGEN AND ACCUMULATION OF LIPOIDS, BY "KIZAKI-KO-MASU", *ONCORHYNCHUS MASOU*, BREVOORT, IN LARVAL AND POST-LARVAL STAGES.

By

Hidesaburo Sekine, assisted by Yuji Kakizaki.

(Received June. 6th., 1930)

"Kizaki-ko-Masu" or the common trout-landlocked were employed as experimental animals on this study was carried out at the Fishculture Station by the Lake of Kizaki, the Prefecture Nagano. 1000 eggs 37 days old after fertirization were divided into two parts in the same number, one of them fed in the hatchery (in the shade) and the other kept out of it (in the light), and the both fed on the same diet to see what effects are there.

78 and 88 larvae just absorbed their yolk-sacs in the both groups were respectively taken for the first analytical samples on the 18 th. in February –the 25th. day from the beginning.

TABLE I. The influence of the light on the larvae just absorbed their yolk–sacs.

	Live weight	Water	Solids	Ash	Calcium	Organic matter	Nitrogen
Shade	296.7	247.9	48.82	3.642	0.923	45.18	5.37
	100.0	83.55	16.45	1.216	0.310	15.23	1.81
			100.00	7.46	1.89	92.54	11.00
Light	291.7	241.5	50.245	3.792	0.959	46.45	5.64
	100.0	82.78	17.22	1.300	0.329	15.90	1.93
			100.00	7.55	1.91	92.45	11.23

Table I indicates the analytical results of the samples mentioned above. These figures indicate that the sun–light brings already a sufficient effect chemically on young fry through the larval stage. By comparision with those fishes are shown in the table, the ones in the shade are havier in live weight but less in solids (both organic and inorganic, especially N & Ca) than those in the light. And these results coinside to the preceding report on fasting of salmons, *Oncorhynchus keta* Walbaum, in the light and dark.

The second examination was done on the 89th. day (May 18th.) since the first examination had been done. According to the results (see Table II) it may be seen that the light brings the better effects on both growth and number of the fishes survived.

TABLE II. The influence of the sun–light on the feedings and samples for chemical analyses.

In the shade	Live weight (g.)	Number	Average weight	Samples for analyses.* Number	Samples for analyses.* Average weight
Total weight	167	121	1.38		
The items					
Large	57	30	1.9	10	2019.4
Medium	76	55	1.38	22	1230.2
Small	34	36	0.943	19	788.0
In the light					
Total weight	254	247	1.03		
The items					
Large (specially)	20	4	5.0	4	3950
Large	48	30	1.6	15	1401
Medium	177	195	0.908	20	849.7
Small	9	18	0.5	18	483.05

It is noticable phenomenon that there are remarkable differenciations in the rates of growth of the fishes feeding on the same condition, and it may be seen that the larger ones, as comparing with the smallers, rich generally in lipins but poor in nitrogen, as shown in Tables III and IV

* Samples for analyses of the study are weighed after took off surface water of their bodies.

TABLE III. Results of analyses of the second examination.

In the shade	Live weight (g)	Solids	Ash	Calcium	Nitrogen	Phos-phorus	Lipoids
Large	2019.4	470.68	34.09	5.264	36.16	6.206	171.3
	100	23.349	1.725	0.261	2.255	0.357	8.48
		100	7.40	1.12	9.657	1.524	36.33
Medium	1230.2	262.95	21.94	2.682	25.17	4.583	80.89
	100	21.38	1.783	0.218	2.046	0.373	6.58
		100	8.34	1.02	9.57	1.743	30.76
Small	788.0	160.3	13.89	2.229	17.04	2.526	40.51
	100	20.35	1.762	0.283	2.163	0.332	5.15
		100	8.66	1.39	10.63	1.631	25.30
In the light							
Large (specially)	3950	949.5	80.48	17.68	99.31	15.52	312.3
	100	24.04	2.037	0.448	2.514	0.392	7.91
		100	8.475	1.86	10 46	1.635	32.89
Large	1401	314.1	26.56	4.70	29.77	5.075	107.3
	100	22.42	1.895	0.336	2.135	0.362	7.66
		100	8.435	1.49	9.48	1.616	34.15
Medium	849.7	181.25	13.74	3.62	18.25	1.215	56.87
	100	21.33	1.62	0.427	2.147	0.143	6.69
		100	7.58	2.00	10.07	0.670	31.40
Small	483.1	96.28	7.18	2.032	11.00	1.414	28.28
	100	19.95	1.49	0.421	2.278	0.210	5.86
		100	7.45	2.11	11.42	1.052	29.36

The average values of these samples are calcurated and concised in Table IV.

On the average weights of the fishes in the shade and in the light the former is greater than the latter, and the former accumulates more lipoids but less in calcium assimilation.

TABLE IV. The average chemical compositions of all the samples in the both conditions.

	Live weight (mg.)	Solids	Ash	Calcium	Nitrogen	Lipoids
Shade	1258	251.2	22.15	3.118	26.80	93.58
Light	938.5	201.4	14.86	3.810	19.28	63.53
Shade	100	21.56	1.762	0.248	2.130	7.439
Light	100	21.46	1.647	0.406	2.161	6.767

The last examination was done on the 120th. day (September 14–15th.) with the similar results to those in the second time, as are shown in Table V.

TABLE V. The influence of the sun–light on the feedings and the samples for analyses.

	Shade			Light		
	Live weight (g.)	Number	Average weight (g.)	Live weight (g.)	Number	Average weight (g.)
Total weight	183.98	30	6.133	277.86	77	3·73974
The items						
Large	60.38	7	8.628	90.75	14	6.482
Medium	95.84	16	5.990	122.07	33	3.6991
Small	27.76	7	3.966	52.08	30	1.736

The medium fishes in the former and the large ones in the latter both of which are almost the same weight were analysed, and the results are given in Table VI. According to the figures the fishes in the light, as compared with those in the shade are rich in solids, ash, calcium, magnesium and nitrogen while are poor in lipoids and lipoids–nitrogen.

TABLE VI. Analytical results of the all fishes in the both condition on the end.

	Live weight (mg.)	Solids	Ash	Calcium	Magnesium	Nitrogen	Lipoids	N in lipoids
Shade	5990	1391	115.3	21.96	1.976	141.4	427.6	2.103
	100	23.22	1.924	0.3665	0.033	2.360	7.139	0.035
		100	8.288	1.578	0.142	10.148	30.74	0.151
Light	6482	1437	139.9	23.96	4.342	153.8	339.8	0.956
	100	25.33	2.466	0.4224	0.043	2.712	5.991	0.0174
		100	9.735	1.667	0.171	10.725	23.65	0.0686

Summary and Conclusion.

1) The influence of the sun–light on the eggs, larvae and fry of Oncorhynchus masou (Brevoort) are studied.

2) The intensity of the sun–light may be effected already upon their chemical compositions in the fry through the oval and larval stages. The fish fed in the lighter place (in the open air out of the hatchery), as compared with the darker place (in faint light inside of the house), are rich in solids, inorganic matters (especially calcium) and nitrogen and are poor in fatty substances.

3) There occur the considarable differenciation in weights of the fish in a group fed on the same diet regardless of the light. And the larger ones are in generally rich in solids especially fatty substances.

4) From these facts, it is concluded as follows :– This species of fishes *Oncorhynchus masou* (Brevoort) may be already fond of the light place on the youngest days. The fish therefore fed in the open air assimilates the more

calcium and nitrogen while that in doors accumulates surplus bodily fats,— these results are the similar to the cases in the preceding report on fasting of salmons fry *Oncorhynchus keta* (Walbaum[1]) in light and darkness.— and the larger ones in a group accumulate in generally the more debot fats without regard of the qauntity of the light.

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(1) H. Sekine (1929): Biochemical Studies on Salmonidae. IV. Bul. of the Agr. Chem. Soc. of Japan. Nos. 61–63, 63.

BIOCHEMICAL STUDIES ON *SALMONIDAE*. VI.

ON THE SUN-LIGHT ON CHEMICAL COMPOSITION OF A FISH. II. ESPECIALLY ON ASSIMILATION OF CALCIUM AND NITROGEN AND ACCUMULATION OF LIPOIDS BY RAINBOW TROUT'S FRY, *SALMO IRIDIUS* GIBBON.

By

Hidesaburo Sekine, assisted by Yuji Kakizaki.

(Received June. 6th., 1930)

The same experiments as had been reported in the preceding paper were repeated with the rainbow trout.

2000 young fry of rainbow trouts, their yolk-sacs just absorbed on the end of June, were divided into two groups which kept inside and outside of the hatchery at Kizaki, Nagano. The fry in the same stage were analyzed and their chemical compositions are illustrated in the following table.

Table I. Chemical compositions of rainbow trouts soon after absorbed their yolk-sacs.

Live weight (mg.)	Solids	Ash	Calcium	Magnesium	Nitrogen	Lipoids	N. in-lipoids
150	26.67	1.846	0.166	0.9365	3.255	3.865	0.0781
100	17.68	1.231	0.111	0.0244	2.170	2.576	0.0414
	190.00	6.964	0.626	0.1377	12.296	14.566	0.2340

On the 14–15th. in September (the 69–70th. day) all the fishes surviving the full experimental period were caught for examination the results of which are

summarized in Table II.

TABLE II. The influence of the sun–light on the fishes in the different conditions, and samples for analyses.

	In the shade			In the light		
	Live weight (g.)	Number	Average weight	Live weight (g.)	Number	Average weight
Initial	152	1000	0.852	150	1000	0.150
Final	227	492	0.461	240	357	0.695
Samples for analysis;						
Large	35.849	42	0.8726	42.340	50	0.8468
Small	87.620	196	0.4470	46.580	100	0.4658

The two kinds of analytical samples weighing almost the same in averages as shown in Table II. The results of the analyses are summarized in Table III.

TABLE III. The chemical compositions of the rainbow trouts fry which fed inside and outside of the hatchery for about 70 days.

In the shade	Live weight (mg.)	Solids	Ash	Calcium	Magnesium	Nitrogen	Lipoids	N in lipoids
Large	853.5	180.5	10.88	1.194	0.249	19.05	50.21	0.1408
	100.0	21.14	1.276	0.1399	0.0291	2.232	5.88	0.0165
		100.00	6.030	0.6614	0.1377	10.554	27.82	0.0780
Small	545.3	107.05	7.134	0.733	0.137	10.72	26.40	—
	100.0	19.65	1.308	0.128	0.0251	1.966	4.84	—
		100.00	6.657	0.6838	0.1277	10.006	24.64	—
In the light								
Large	846.8	171.45	10.73	1.279	0.228	18.66	44.47	0.0849
	100.0	20.24	1.267	0.1509	0.0270	2.204	5.252	0.0100
		100.00	6.257	0.7437	0.1331	10.884	25.884	0.0496
Small	465.8	85.42	6.267	0.635	0.131	9.01	17.73	—
	100.0	18.34	1.346	0.1364	0.0282	1.979	3.806	—
		100.00	7.337	0.7437	0.1537	10.790	20.75	—

Discussion of Result.

Comparing the fishes in the hatchery with those in the open air, the former is lighter in total and average weights but is greater in number of fishes survived. In the next, on their chemical compositions the former contains the more solids, lipoids and lipoids–N but the less ash, calcium, and nitrogen than the latter. It may, therefore, be seen that the majourties of the results are similally to the preceding report(1).

Conclusion.

The twice experiments on the influence of the sunlight on feeding fishes

and their chemical compositions may be concluded as follows :–

Keeping trouts (in the youngest days) in the different conditions (in the inside and out of the hatchery) should be brought the different metabolisms in their bodies.

Then the fish in the open air assimilates high content of calcium and nitrogen while that in the shade contain smore deposit fats. Therefore, within the limits of general condition of feeding trouts, the more light brings the more assimilation of calcium and nitrogen but the less deposition of fatty substances. And the former case shows generally high value of total weight of the trouts survived than that of the latter.

According to the experimental results, it may be provable that it is not good to keeping the larvae and the young fry in the hatchery, which is always gloomy, for a long time after they have been hatched out.

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(1) H. Sekine :– Biochemical Studies on Salmonidae. V., Bul. of the Agr. Chem Soc. of Jap., (1930)

THE IODOMETRIC DETERMINATION OF FURFURAL.

By

Shuiku Sasaki.

(Received 5th , July, 1930.)

Among many methods determining furfural Pervier and Gortner's bromine method has called to the author's attention. The method is as follows :——— To the acid solution of furfural, some potassium bromide is added and titrated by N/10 potassium bromate. The end point of the reaction between bromine and furfural is determined by the time factor of colour change or by the degree of deflection of the needle of a galvanometer. This method is not reliable for they did not taken into account the effect of temperature upon the reaction. According to our experiments, bromine combines with more furfural at high temperature than at low temperature. Consequently, we modified their bromine method to an iodine method, and got more satisfactory results from the point of view of accuracy.

Method :—— Take about 6 per cent hydrochloric acid solution containing 5 to 18 mg. of furfural in 100 c.c. in a bottle, such as Merk's sulphuric acid bottle, which has a capacity from 250 to 300 c.c. Add 5 c.c. of 2 per cent potassium bromide, and exactly 10 c.c. of N/10 potassium bromate solution (containing 2.7838 g. potassium bromate in 1 litre of water). Seal tightly with a glass stopper. Allow it to stand for just 2 hours in a thermostat. Then add 10 c.c. of potassium iodide and titrate with N/50 sodium thiosulphate solution, of which 50 c.c. is equivalent to 50 c.c. of N/50 potassium bromate solution or to 10 c.c. of N/10 potassium bromate solution formerly added. When the end of the reaction approaches the brown colour of the solution changes to a pale yellow colour. At this time 2 c.c. of 0.5 per cent starch solution is added, and the titration is continued drop by drop, shaking the solution vigorously until the point of complete colourlessness is reached.

The following table shows the number of c.c. of N/50 potàssium bromate which combines with 1 mg. of furfural at each temperature.

Temp.	Bromate, c.c.	Temp.	Bromate, c.c.
15	2.017	27.5	2.226
17.5	2.078	30	2.261
20	2.122	32.5	2.313
22.5	2.156	35	2.357
25	2.191		

Example :—— When the temperature of the thermostat shows 20°, and 30 c.c. of N/50 sodium thiosulphate solution is required, the quantity of furfural is calculated as follows :——

$$50 \text{ c.c.} - 30 \text{ c.c.} = 20 \text{ c.c.}$$
$$2.122 \text{ c.c.} : 1 \text{ mg.} = 20 \text{ c.c.} : X \text{ mg.}$$
$$X = 20/2.122 = 9.42 \text{ mg. furfural.}$$

LITERATURE.

(1) Pervier and Gortner : The estimation of pentoses and pentosans. II. The determination of furfural. J. Ind. Chem., **15**, 1255, 1923.

THE RESPIRATORY PROCESS OF THE SILK WORM AS AFFECTED BY TEMPERATURE, MOISTURE AND AIR CURRENT.

By

Otomatsu Fujii.

(Received 10th, July 1930)

(1)

The silk worm is extremly sensitive to slight changes in its environment, especially temperature, moisture, and air current. Yet, despite the many papers dealing with this worm upon these factors, quantitative studies of its relations to the respiratory process have been rare and limited in scope.

(2)

Several years ago Crozier[1] reported the use of the critical thermal increment (μ) for the characterization of biological process in various plants and animals whose velocities are a function of temperature and pointed out that in the case of oxidative phenomena critical increments were characteristically found to be of two, possibly three, types: $\mu=11,500$ and 16,100 or 16,700, and the first was commonly encountered above 15°C, the second below that temperature, but these relations might be reversed.

The author studied these relations of the respiratory process to other factors under as constant conditions as possible.

As shown in the following results, the respiratory process as affected by changes in temperature between the upper and the lower limit can be described experimentally in the terms of the follwing experimental equation

$$y=kt^p$$

in which y is the amount of carbon dioxide produced, t is the temperature, k and p are the constant.

TABLE I.

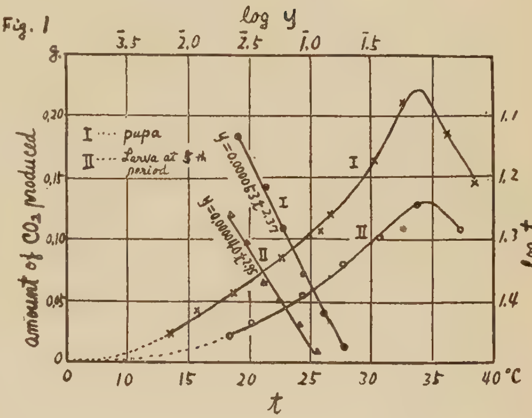
CO_2 production of pupa at different temperature.

t °C	CO_2 g.	$\mu=\frac{4.61\,T_1T_2(\log k_2-\log k_1)}{T_2-T_1}$	$k=\frac{y}{t^{2.37}}$	$p=\frac{\log\frac{y}{0.000053}}{\log t}$
13.4	0.0246	19981	0.000052	2.37
16.3	0.0416	20694	0.000056	2.39
19.2	0.0593	18068	0.000054	2.38
22.6	0.0842	15986	0.000052	2.36

26.9	0.1239	16844	0.000051	2.36
30.2	0.1681	22784	0.000052	2.37
32.1	0.2124	−7054	0.000057	2.39
36.2	0.1822	−19772	——	——
38.4	0.1454	——	——	——

From above results we see that the upper limit of the respiratory process is at about 33∼34°, and the lower limit at about 7∼8°, and the μ values suggested by Crozier are not constant. In his view, the same reaction exhibits different temperature coefficients when activated by different catalysts, and so the respiratory process must be activated by many different catalysts. But is this true? Further studies will solve these relations.

Fig. 1



(3)

The relations of moisture to the respiratory process can also be shown in terms of the following experimental equation

$$y=km^{-p}$$

in which y is the amount of the carbon dioxode produced, m is the relative humidity, k and p are the constant. The results were as follows.

TABLE. II.

CO_2 production of larva at different degrees of moisture.

No.	Live weight	t	m	CO_2 produced: measured	CO_2 produced: calculated ($y=0.213\,m^{-0.075}$)
	g.	°C	%	g.	g.
♂35	101.6	24.4	71	0.1559	0.1551
♀35	101.6	24.5	78	0.1544	0.1540
〃	101.6	24.4	83	0.1531	0.1532
〃	101.6	24.4	98	0.1522	0.1513

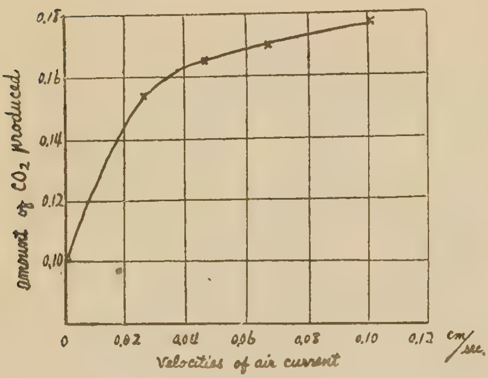
(4)

The continuous effects of temperature and moisture in the respiratory process were examined. As above mentioned, it is affected by temperature in the terms of t^p, in which p is positive. But if observations are made during a continuous period it will be found that the higher the temperature the more rapid the decrease.

In the case of the effect of moisture, the same result was obtained.

(5).

The effect of the velocities of air current on the respiratory process was studied as far as possible at the same temperature and moisture. The following diagram shows the amount of carbon dioxide produced at the various velocities of air current.



Effect of velocities of air current on the respiratory process of larva at 5th period.

From this diagram we see that when there is no air current the amount of carbon dioxide produced is very much diminished, but as the air current increases the amount of carbon dioxide also increases, up to 0.1 cm/sec., the limit of our experimental apparatus.

Summary.

(1) The effects of same environmental conditions on the respiratory process in the silk worm's pupa and larva at 5th period were studied.

(2) The effects of temperature, in the rang 14～32°C, and of moisture in the range 60～100%, can be described in terms of the following experimental equations.

$$y = kt^{p}$$
$$y = km^{-p}$$

in which y is the amount of carbon dioxide produced, t is the temperature, m is the moisture, k and p are the constant.

(3) The upper critical temperature of the respiratory process is about 33～34°C, and its lower critical temperature is probably about 7～8°C.

(4) When there is the same temperature or moisture during a continuous period, the higher the temperature or the greater the moisture, the more rapid is the decrease in the production of carbon dioxide.

(5) Under the condition of no air current carbon dioxide production is very much diminished and it is increased up to 0.1 cm/sec. in proportion to the velocity of the air current.

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ISOLATION OF XANTHOPHYLL FROM FRESH GREEN LEAVES.

By

K. SUZUKI and T. NISHIKAWA.

(Received 13th. July 1930).

Willstätter and Mieg (Ann. 355, I, 1907) were the first to isolate xanthophyll crystals from green leaves. They secured by their own method a yield of 12 grams of the crude crystals from 100 kgs. of air-dried nettles leaves. Jörgensen and Stiles (New Physiologist, Reprint, 1∼180, 1917) isolated by another method 0.8 grams of the crude crystals of xanthophyll from 2 kgs. of air-dried nettle leaves.

According to these methods, fresh leaves must be first dried carefully either in the shade or by using some special rapid-drying apparatus which is fitted for drying of a considerable amount of fresh leaves in a short time and more over not getting the temperature above 40°C, since too high a temperature destroys a large percentage of the pigments present in the leaves.

Sometimes, these drying processes are very inconvenient, and hence we attempted to isolate xanthophyll crystals directly from fresh green leaves and obtained the satisfactory result by the following simple method.

5.1 kgs. of the fresh leaves of radish (Raphanus sativus, L.) are chopped about 1 cm. in length, divided into some portions and then put separately into an enamelled pot. Each portion is heated for a few minutes above 70°C with alcohol which is allowed to soak into the chopped fresh leaves. By this treatment, the fresh green leaves lose some parts of its moisture and will be changed to be macerated easily.

After heating, the alcohol is pressed off and the heated leaves are macerated in a motor. The alcohol which has been used for the heating of the first portion of the chopped fresh leaves can be used three successive treatments of heating of the fresh leaves, but not further.

Pigments are extracted from the macerated leaves with ether and the ether extract quickly sucked away. The extraction is repeated three times using fresh ether each time. By the extraction process, above mentioned, all of the leaf pigments will be extracted and the macerated leaves on the filter will be colorless. The ether extract is washed out with distilled water in order to remove traces of the alcohol, and dried over anhydrous sodium sulfate and then filtered.

The alcoholic solutions which were obtained by the heating of the chopped fresh leaves at the first treatment of this investigation are now combined, mixed with about equal volume of ether and, if neccessary, small amount of water is added at this time. The whole is shaken thoroughly and a great amount of water is added in order to cause a separation of the ether layer completely, and the aqueous alcohol layer which contains the brownish-yellow flavones is drawn off. The ether solution is washed out with water several times until no more green color is extracted, and dried over anhydrous sodium sulfate, then filtered.

All the ether solutions of the pigments are now combined, saponified with a small amount of methyl alcoholic potash by shaking thoroughly.

After complete saponification of chlorophylls, the ether solution is washed several times with water to remove the alkali-chlorophyllines completely. From this solution, ether is now evaporated in vacum at a room temperature. If reddish-green fluorescence is observed in the solution with the evaporation of the ether, a small amount of methyl alcoholic potash must be added again in order to saponify traces of chlorophylls, and the mixture is shaken well, and then the ether solution is washed several times with distilled water until the green color is completely removed. Then the ether is entirely evaporated off in vacuum at a room temperature, there remain xanthophyll crystals with a large quantity of reddish-brown resinous substances and some amount of carotin crystals.

About 500 c.c. of the light petroleum ether (b.p. 35～55°C) is poured to the residue and stirred well. On standing over a night at a room temperature, all the reddish-brown resinous substaces and the carotin crystals are dissolved but xanthophyll remains insoluble in the petroleum ether.

The xanthophyll crystals are separated by the filtration, washed with a little quantity of the petroleum ether, and then recrystalized twice from boiling absolute methyl alcohol.

We have obtained by this method 0.255 gram of pure xanthophyll crystals whose melting point is 174～175°C (uncorrected) from 5.1 kgs. of the fresh radish leaves.

The moisture content of the fresh radish leaves which were used for this investigation was 84.615% and that of air-dried state 2.955%.

Therefore, 5.1 kgs. of the fresh leaves can be converted into 935 grams of air-dried state. Consequently, the yield of 0.255 gram of the pure xanthophyll crystals from 5.1 kgs. of the fresh leaves must be said to be satisfactory result.

STUDIES ON THE SAPONIN OF SOY-BEAN. PART II.

By

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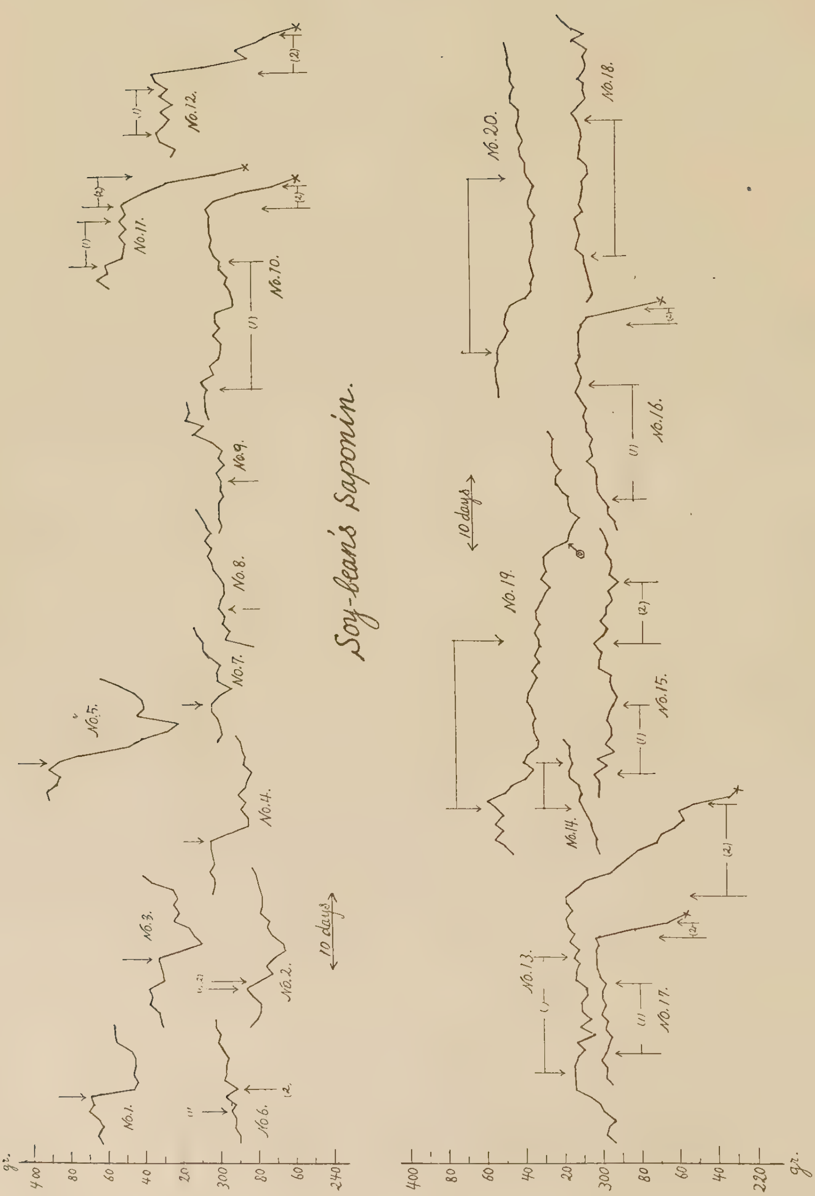
(Received 13 th., July 1930)

The author has received fortunately a large quantity of the alcoholic extraction of soy-bean from "Mantetsu Chūō Shikenjo", to which the author desires to express his thanks, and has been able to isolate the saponin easily as crystals. The Na–salt of saponin, decomposing at 259∼260°, is prepared as hexagonal plate by neutralisation with Na–hydroxide from 70% alcoholic solution of free saponin.

The haemolytic and toxic powers of the saponin of soy-bean are studied comparing with Merck's saponin (pur. albiss) (aus der Wurzer der levantischen Saponaria).

The haemolytic power (against horse's blood) of the free saponin of soy-bean is observed in the concentration of 2000 : 1 and that of Na–salt is observed in the concentration of 50000 : 1, but the corpuscle is not destroyed completely in so high concentration such as 200 : 1. On the contrary Merck's saponin shows its haemolytic power in the concentration of 50000 : 1 and in the concentration of 25000 : 1 shows complete haemolysis.

The toxic power of soy-bean's saponin (free and Na–salt also), examined on pigions, is very weak though not being quite harmless, but that of Merck's saponin is very violent and a pigion dies by the hypodermic injection of 0.03 g. or by each deglutition of 0.05 g. during 2 days.


Soy-bean's Saponin.
10 days
10 days
gr.
gr.
No. 1.
No. 2.
No. 3.
No. 4.
No. 5.
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400
80
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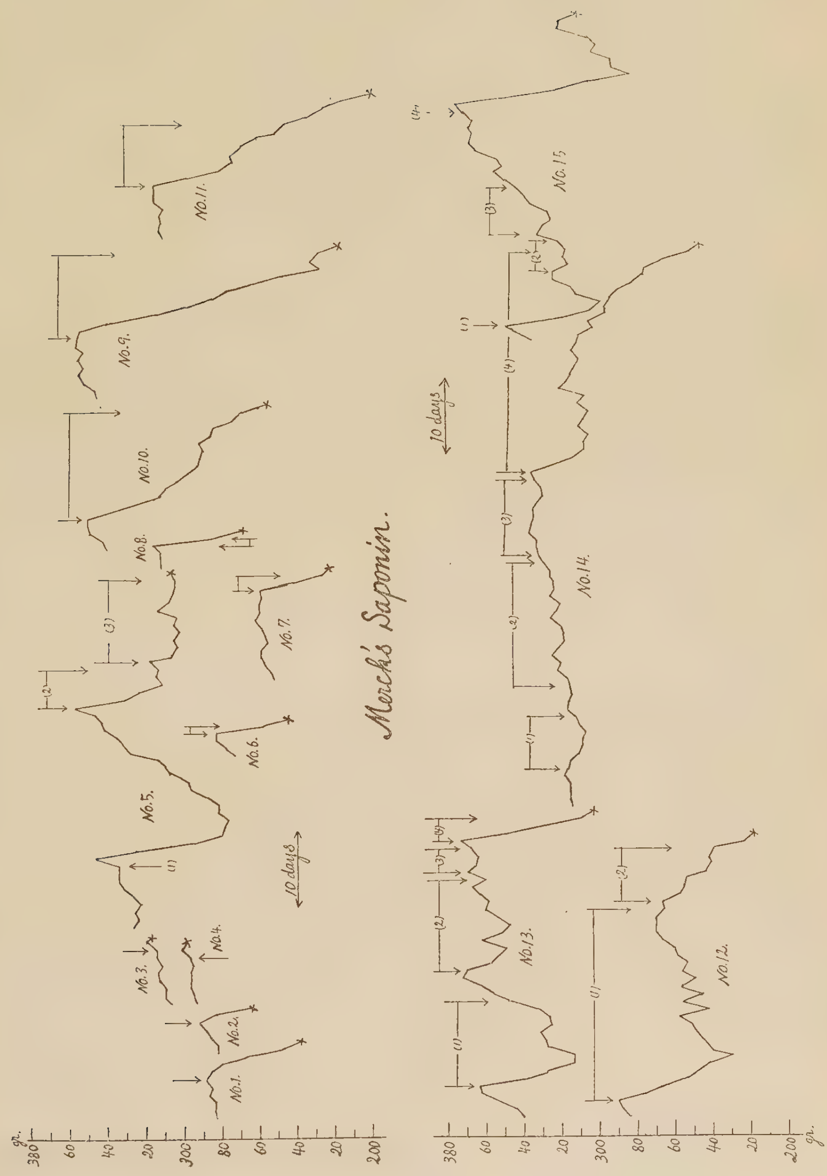
Fig 1.

Fig 2.


Merck's Saponin.
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No.14.
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gr.

THE DISTRIBUTION OF PHOSPHORUS IN COW'S MILK AND THE SCHEME FOR THE SEPARATION OF PHOSPHATIDES

By

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(Received 9th September 1930)

A. The Distribution of Phosphorus in Cow's milk

In order to extract the phosphatides, it is necessary first to determine the distribution of phosphorus in cow's milk. Liquid milk can not be extracted with ether or with other organic solvents owing to its large content of water.

The substance applied in this experiment is the milk powder, dried by the Buflovak drum drier below 70°C. Its content of moisture is 4.498%, determined by the method of drying in the air bath of 105°C.

The content of total phosphorus was determined[5] in the ash of sample which was burned with a little of fusing mixture.

	In 100 g. of milk powder	In 100 g. of dry matter
Total phosphorus	0.745 g.	0.781 g.

The amounts of phosphorus, which are soluble in the various organic solvents, were determined by extracting with three solvents in the following orders.

(A) Ether – Acetone – Alcohol
(B) Acetone – Ether – Alcohol
(C) Alcohol – Ether – Acetone

Experiment (A)

1) At first a pair of each 10 g. of sample was extracted with ether by the Soxhlet's apparatus for a week. After the extraction had been completed, the extract was freed from ether and weighed.

	In 100 g. of milk powder	In 100 g. of dry matter
Total ether soluble matters	3.609 g.	3.779 g.

2) The above extract was dissolved in a very little of ether. On adding of acetone to the ethereal solution, the white amorphous precipitates were produced. The precipitates were filtered through the filter paper and washed with acetene.

Then the acetone soluble matter was freed from acetone and weighed.

It was burned to ashes with a little amount of fusing mixture and the content of phosphorus was determined.

	In 100 g. of milk powder	In 100 g. of dry matter
Ether and acetone soluble matters	3.078 g.	3.223 g.
Ether and acetone soluble P	0.10 mg.	0.105 mg.

The ether and acetone soluble fraction contains 0.003% of phosphorus. Then it might be assumed that this fraction was contaminated with about 0.082% of phosphatides, assuming that the phosphatides contain 3.94% of phosphorus as the mixture of equal parts of dipalmityl–, distearyl–and dioleyl–lecithin.

The ether soluble but acetone insoluble matter was dissolved in ether, and filtered through the filter paper. After it was freed from ether and weighed, it was burned to ashes with a little of fusing mixture, and the content of phosphorus was determined.

	In 100 g. of milk powder	In 100 g. of dry matter
Ether soluble but acetone insoluble matters	0.531 g	0.556 g.
Ether soluble but acetone insoluble P	0.07 mg.	0.073 mg.

This fraction contains 0.013% of phosphorus, as phosphatides 0.332% assuming that the content of phosphorus in phosphatides is about 3.94% as the mixture of lecithins.

3) The residue which was completely extracted with ether was freed from ether, and was again extracted with acetone at 30∼40°C by the Soxlhet's apparatus for 4 days.

After the extracts were freed from acetone, it was weighed. Then it was disssolved in a little amount of ether. The insoluble matter was separated and washed with ether and acetone. Acetone was added to the ethereal solution and divided into two parts, that is, the one is soluble in both ether and acetone, and the other is soluble in ether but insoluble in acetone. The residue which is insoluble in both ether and acetone, was dissolved in water.

The above three fractions were weighed and the contents of phosphorus were determined by the same method as the above process.

	In 100 g. of milk powder	In 100 g. of dry matter
Ether and acetone soluble matters	11.443 g.	11.982 g.
Ether and acetone soluble P	0.53 mg.	0.55 mg.
Ether soluble but acetone insoluble matters	0.580 g.	0.607 g.

Ether soluble but acetone insoluble P	0.33 mg.	0.35 mg.
Water soluble matters	1.629 g.	1.706 g.
Water soluble P	0.64 mg.	0.67 mg.
Total soluble matters	13.652 g.	14.295 g.

4) The residue which was extracted with ether and acetone, was further extracted with the boiling absolute alcohol.

The alcoholic solution was evaporated to a little volume and extracted with ether. Much insoluble matter was remained, which was further extract-ed with acetone and alcohol. Total extracts were mixed and evaporated to a little volume and expelled almost all of the solvents. And again it was dissolved in ether. There remained some insoluble matter.

The clear ethereal solution was divided into two parts, that is, the one is soluble in both ether and acetone, and the other is soluble in ether but insoluble in acetone, by the same process as the above experiment. Total residue which is insoluble in all of ether, acetone and alcohol, was soluble in water.

	In 100 g. of milk powder	In 100 g. of dry matter
Ether and acetone soluble matters	0.423 g.	0.443 g.
Ether and acetone soluble P	4.11 mg.	4.30 mg.
Ether soluble but acetone insoluble matters	0.121 g.	0.127 g.
Ether soluble but acetone insoluble P	0.33 mg.	0.35 mg.
Water soluble matters	17.275 g.	18.089 g.
Water soluble P	16.97 mg.	17.76 mg.
Total soluble matters	17.819 g.	18.658 g.
Total soluble P	21.41 mg.	22.42 mg.

Experiment (B)

1) At first a pair of each 10 g. of sample was extracted with acetone by the Soxhlet's extracting apparatus for 4 days. After the extraction had been completed, the extracts were freed from acetone and weighed.

	In 100 g. of milk powder	In 100 g. of dry matter
Total acetone soluble matters	15.988 g.	16.741 g.

2) The above extract was dissolved in a very little of ether. On addition of acetone to the ethereal solution, the white amorphous precipitates were produced. The precipitates were filtered through the filter paper and washed with acetone. Then the acetone soluble matter was freed from acetone and weighed. It was burned to ashes with a little of fusing mixture and the content of phosphorus was determined.

	In 100 g. of milk powder	In 100 g. of dry matter
True acetone soluble matters	13.720 g.	14.366 g.
True acetone soluble P	1.05 mg.	1.10 mg.

The true acetone soluble fraction contains 0.008% of phosphorus. Then it might be assumed that this fraction was contaminated with about 0.194% of phosphatides.

The residues from the acetone soluble matter were dissolved in ether, and filtered through the filter paper. After it was freed from ether and weighed, it was burned to ashes with a little of fusing mixture, and the content of phosphorus was determined.

	In 100 g. of milk powder	In 100 g. of dry matter
Ether soluble but acetone insoluble matters	0.829 g.	0.868 g.
Ether soluble but acetone insoluble P	0.75 mg.	0.79 mg.

This fraction contains 0.090% of phosphorus, as phosphatides 2.296% assuming that the content of phosphorus in phosphatides is 3.94%.

The residue which was insoluble in acetone and ether, was dissolved in water. After it was evaporated, its weight and the content of phosphorus was determined.

	In 100 g. of milk powder	In 100 g. of dry matter
Water soluble matters	1.439 g.	1.507 g.
Water soluble P	0.38 mg.	0.40 mg.

Almost all of this fraction may be sugar and inorganic substances.

3) The milk powder which was completely extracted with acetone, was freed from solvent by evaporating at 80°C and powdered. Then this was again extracted with ether by the Soxlhet's extracting apparatus for 3 days. After the extract was freed from ether, it was weighed. The content of phosphorus in this fraction was determined as the above process.

	In 100 g. of milk powder	In 100 g. of dry matter
Ether soluble matters	0.243 g.	0.254 g.
Ether soluble P	0.05 mg.	0.05 mg.

This fraction contains only 0.021% of phosphorus and 0.523% as phosphatide.

4) The residue which was extracted with acetone and ether, was further extracted with the boiling absolute alcohol.

The alcoholic solution was evaporated to a little volume and extracted with ether. Much insoluble matter was remained, which was further extracted with acetone and then with alcohol. Total extracts were mixed and evaporat-ed to a little volume and again it was dissolved in ether. There was

remained some insoluble matter.

The clear ethereal solution was divided into two parts, that is, the one is soluble in both ether and acetone, and the other is soluble in ether but insoluble in acetone, by the same process as the above experiment. Total residue, that is insoluble in all of ether, acetone and alcohol, was soluble in water.

	In 100 g. of milk powder	In 100 g. of dry matter
Ether and acetone soluble matters	0.253 g.	0.265 g.
Ether and acetone soluble P	4.12 mg.	4.31 mg.
Ether soluble but acetone insoluble matters	0.105 g.	0.110 g.
Ether soluble but acetone insoluble P	0.10 mg.	0.10 mg.
Water soluble matters	19.077 g.	19.975 g.
Water soluble P	17·82 mg.	18.66 mg.
Total soluble matters	19.435 g.	20.350 g.
Total soluble P	22.04 mg.	23.04 mg.

Experiment (C)

1) A pair of each 10 g. of milk powder was extracted with the boiling absolute alcohol for five times. The extract was clear but faintly colored with yellowish green. On cooling, much white amorphous substance was precipitated. The precipitates were separated by centrifusing and washed with alcohol.

2) Precipitates: The precipitates were dissolved in ether. At that time, only a little of insoluble matter was remained, and it was soluble in water. On adding acetone in the clear ethereal solution, the white amorphous substance was precipitated. This was separated and washed with acetone.

Thus, the white precipirates were dissolved into three parts, that is, the one which is soluble in ether but not in acetone, the one which is soluble in both ether and acetone, and one which is insolubie in both ether and acetone but soluble in water.

	In 100 g. of milk powder	In 100 g. of dry matter
Ether and acetone soluble matters	8.469 g.	8.868 g.
Ether and acetone soluble P	0.17 mg.	0.18 mg.
Ether soluble but acetone insoluble matters	1.047 g.	1.096 g.
Ether soluble but acetone insoluble P	0.15 mg.	0.16 mg.

Water soluble matters	1.449 g.	1.517 g.
Water soluble P	0.91 mg.	0.95 mg.
Total soluble matters	10.965 g.	11.481 g.
Total soluble P	1.23 mg.	1.29 mg.

3) Cold alcohol soluble matters: The alcoholic solution was evaporated to a little volume and extracted with ether. The ethereal solution was freed from ether and again dissolved in ether. A little of insoluble matter was remained.

The clear ethereal solution was divided into two parts, that is, the one is soluble in both ether and acetone, and the other is soluble in ether but insoluble in acetone, by the same process as the above experiment.

The white syrupy matter that is insoluble in all of ether, acetone and alcohol, was soluble in water.

	In 100 g. of milk powder	In 100 g. of dry matter
Ether and acetone soluble matters	5.999 g.	6.282 g.
Ether and acetone soluble P	5.03 mg.	5.27 mg.
Ether soluble but acetone insoluble matters	0.093 g.	0.097 g.
Ether soluble but acetone insoluble P	0.52 mg.	0.54 mg.
Water soluble matters	18.693 g.	19.573 g.
Water soluble P	17.72 mg.	18.55 mg.
Total soluble matters	24.785 g.	25.952 g.
Total soluble P	23.27 mg.	24.37 mg.

4) The residue from the extraction with the boiling alcohol, was further extracted with ether by the Soxhlet's extracting apparatus for a week, and then with acetone for 4 days. There was no extractive matter.

Conclusion

The above three experimental results are summarized as follows:

Solvents	In 100 g. of milk powder					
	Experiment(A) Ether – Acetone – Alcohol		Experiment(B) Acetone – Ether – Alcohol		Experiment(C) Alcohol – Ether – Acetone	
	Substances (g.)	P (mg.)	Substances (g.)	P (mg.)	Substances (g.)	P (mg.)
Ether and acetone soluble	15.648	4.96	14.631	5.41	15.149	5.44
Ether soluble but acetone insoluble	1.290	0.76	1.232	0.94	1.194	0.70
Water soluble	19.794	18.44	21.482	19.06	21.091	19.51
Total	36.732	24.16	37.345	25.41	37.434	25.65

If phosphorus, soluble in ether of acetone, is assumed as that of lecithin assuming as the mixture of equal parts of dipalmityl–, distearyl– and dioleyl-lecithin, containing each 3.94% of phosphorus, it is calculated as the following data.

Solvents	In 100 g. of milk powder		
	Experiment (A) Ether – Acetone – Alcohol	Experiment (B) Acetone – Ether – Alcohol	Experiment (C) Alcohol – Ether – Acetone
Ether and acetone soluble matters (True fats)	15.522 g.	14.494 g.	15.011 g.
Ether and acetone soluble matters (Phosphatides)	0.126 g.	0.137 g.	0.138 g.
Ether soluble but acetone insoluble matters	1.271 g.	1.208 g.	1.176 g.
Ether soluble but acetone insoluble matters (Phosphatides)	0.019 g.	0.024 g.	0.018 g.
Water soluble matters	19.326 g.	20.998 g.	20.596 g.
Water soluble matters (Phosphatides)	0.468 g.	0.484 g.	0.495 g.

Based on the above three experimental results, it is known the following evidence. The greater part of phosphatides in cow's milk are more easily soluble in warm alcohol or warm acetone than ether. And phosphatides which can not be extracted by the direct extraction of the milk powder with ether, become soluble in ether when milk powder is extracted with the same solvent, after it is extracted with warm alcohol or warm acetone and then all the solvent is removed. It is known that when all the solvent is removed from the first acetone extract of the milk powder and the resulting residue is again taken up in ether, a good deal of substance is precipitated from the ethereal solution by acetone. And when the milk powder is treated as above described, the same total soluble matter can be obtained even if extracted with three solvents in different orders.

B. Scheme for Separation for Phosphatides

The presence of lecithin and cephalin in cow's milk was noted by many investigaters, however, not identified by the complete analysis, and there is still without any definite evidence as to the presence of other phosphatide. From the nature of the problem, however, we are still without any reliable method on the isolation of the different phosphatides of cow's milk.

Assuming that all of fats and lipoids which were found in various animal tissues and organs are also present in cow's milk, the experimental procedure should be schemed to separate the individual phosphatide in a chemically pure form following to the many procedures[1] that were planned for the investigations of animal tissues and organs. The various steps in the process are as follows :

The milk powder is throughly extracted with ether by the use of the Soxhlet's extracting apparatus. Fats, cholesterol, lecithin, cephalin, myelin, cuorin, cerebrosides, paramyelin, vesalthin, amidomyelin and sphingomyelin are soluble in ether. After the residue of ether extraction is freed from ether, it is extracted with alcohol at 60°C. Then, a part of lecithin, cholesterol, fats, cephalin, cerebrosides, myelin and sphingomyelin that remain insoluble in the preceding extraction with ether, are extracted with hot alcohol while protein, sugar and mineral matter remain insoluble in the residue.

The ethereal solution is concentrated to a small bulk and treated with excess of acetone, which precipitates the greater part of lecithin, cephalin, myelin, cuorin and cerebrosides and a part of paramyelin, amidomyelin, sphingomyelin and cholesterol, leaving fats, cholesterol and vesalthin in solution. The precipitate is redissolved in a small amount of ether and excess of absolute alcohol added. The addition of alcohol causes cephalin cuorin, cerebrosides, myelin, paramyelin, amidomyelin and sphingomyelin (Precipitate C) to separate, while lecithin remains soluble in the mother liquor (D) which is filtered.

On cooling the above warm alcohol extract, the greater part of cerebrosides, myelin and sphingomyelin is separated. This precipitates (A) may contain some part of cephalin, cuorin, paramyelin and amidomyelin. The greater part of lecithin, cholesterol and fats remain in the mothor liquor (B), which is filtered and may contain also some of cephalin.

Treatment of Precipitate (A)

After repeated dissolving and precipitating in alcohol, the precipitate (A) is separated into the ether soluble part and the ether insoluble part. The ethereal solution is treated with excess of acetone and the resulting precipitate (AA) is separated from mother solution (AB) by filtration. The resulting precipitate (AA) is again treated with ether, and an insoluble residue is separated by filtration. The ethereal solution is precipitated by alcohol; and the precipitate (AAA) is separated from the mother solution (AAB) by filtration.

The first ether insoluble residue of precipitate (A) is treated with hot alcohol. The alcohol soluble part is allowed to cool and the resulting precipitate (AC) is separated from the mother solution (AD) by filtration.

Treatment of Mother liquor (B)

The mother liquor (B) is now concentrated under reduced pressure until all the alcohol is removed. The residue is again taken up in alcohol and the insoluble substance (BA) is separated from the soluble part (BB). The

insoluble substance (BA) is now dissolved in a small volume of water containing a little sodium chloride, and extracted with ether by shaking in the separatory funnel.

The ethereal solution is now concentrated under reduced pressure until all the ether is removed, the rèsidue is treated with warm alcohol. The alcoholic solution is cooled down and the precipitate (BAA) which separate out, is separated from the mother liquor (BAB).

Treatment of Precipitate (C)

The combined precipitates of fraction (C) and fraction (AAA) are treated with ether and divided into two fractions of the insoluble part (CA) and the soluble part (CB). The former contains cerebrosides, myelin, sphingomyelin, paramyelin and amidomyelin, and the latter contains cephalin and cuorin. After the fraction (CB) is evaporated to remove ether, extracted with alcohol at 60°C whereby cuorin[2] is contained in the insoluble part (CBA) and cephalin[3] in the soluble part (CBB), and a precipitate separates on allowing the mixture to stand in the cold.

Treatment of Mother liquor (D)

Mother liquor (D) and mother liquor (AAB) are mixed and concentrated under reduced pressure until all the solvent is removed. The residue (DA) is taken up in ether in which sphingomyelin[4] remains insoluble. The ethereal solution (DB) is filtered and excess of absolute alcohol[4] added, which precipitates (DBA) cephalin leaving leithin and other substances in solution (DBB).

Treatment of Solution (BB)

To the mixture of the soluble part (BB), the mother liquor (AB), the mother liquor (BAB) and the mother liquor (DBB), the alcoholic solution of cadmium chloride is added in excess, and the precipitate (BBA)[4] is separated from the mother solution (BBB).

As there is doubt as to the contamination of fats and cholesterol with this precipitate of phosphatides, they are treated with hot acetone. Then the cadmium chloride of lecithin and cephalin remain insoluble, and the acetone solution is allowed to cool when a deposit (BBAc) occured; fat and cholesterol remain soluble in the mother solution (BBAd).

When the cadmium chloride salts of phosphatides is treated with hot ether[4], cephalin cadmium chloride salt is dissolved, in which lecithin cadmium chloride salt remains insoluble. The ethereal solution is filtered off and allowed to cool, and the cephalin cadmium chloride (BBAe) separated from

the ethereal solution by alcohol (BBAf).

The fraction of lecithin cadmium chloride is now extracted with hot benzene[4], the lecithin cadmium chloride is dissolved but amidomyelin remains insoluble. On cooling this extract, a precipitate of paramyelin[4] forms. The filtrate obtained from the above preapitate is treated with excess of alcohol and the resulting precipitate (BBAa) is separated from the mother solution (BBAb).

The fraction of amidomyelin and paramyelin is again treated with hot alcohol and a precipitate (BBAg) is separated from the mother solution (B BAh).

Treatment of Mother liquor (BBB)

The mother liquor (BBB) is combined with the mother liquor (BBAf), (BBAb) and (BBAh) and concentrated under reduced pressure until all the alcohol is removed. The residue is again taken up in alcohol and the insoluble substance (BBBa) separated from the alcoholic solution (BBBb).

Summary.

The distribution of phosphorus in cow's milk (milk powder) has been determined and the existence of much phosphorus in the fraction of phosphatides has been proved.

The experimental procedure has been schemed to isolate the individual phosphatide, assuming that all of fats and lipoids which were found in various animal tissues and organs are also present in cow's milk.

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ON TEA CATECHIN ISOLATED FROM GREEN TEA.

By

MICHIYO TSUJIMURA.

(Received September 8th., 1930).

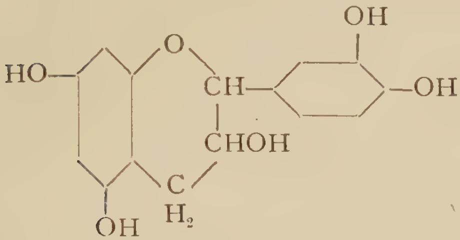
The tannin substance of tea (*Thea sinensis L.*) has frequently been investigated, but no decisive report has yet been pubilished. The auther has recently isolated a catechin from green tea in crystalline state and gave it the name "Tea Catechin". It forms colourless glistening prisms (Fig. 1) when crystallized from aqueous solution and melts at 237~238° (uncorr.) It gives also typical reactions for catechin. The analysis agrees with the formula $C_{15}H_{14}O_6$ which is now generally adopted for catechin. Its specific rotation is: $[\alpha]_D^{25} = -69°$. On acetylation it forms pentaacetyl derivative $C_{15}H_9O(OCOCH_3)_5$ m. p. 151~152°, thus showing the existence of five hydroxyl groups in the molecule. On the methylation, however, tetramethyl compound $C_{15}H_9O(OH)(OCH_3)_4$ m. p. 153~154° is obtained. This indicates that one hydroxyl group can be more difficultly methylated than others. Boiled with a concentrated alkali, phloroglucinol is formed as the chief decomposition product, and when the methylated product is cautiously oxidized by $KMnO_4$, veratric acid is formed. From these observations, tea catechin seems to have close resembrance to *l*-epicatechin isolated from acacia catechu by K. Freudenberg, the only difference being that the former contains no water of crystallization, when dried in the air, while the latter contains four molecules of water under the same condition.(1)

The comparison of tea catechin with other catechins obtained by different authors is as follows :

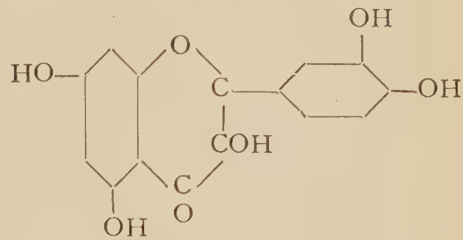
			m. p.	Cryst. water	Secific rotation
A. G. Perkin:	Catechin C	$C_{15}H_{14}O_6$	235~237°	no	no description(2)
M. Nierenstein:	Isoacacatechin	$C_{15}H_{14}O_6$	237~238°	no	inactive(3)
K. Freudenberg:	*l*-Epicatechin	$C_{15}H_{14}O_6$	245° (corr.)	4 mol	$[\alpha]_{Hg}$ yellow in alc. ~69°(1)
The present author:	*Tea* catechin	$C_{15}H_{14}O_6$	237~238°	no	$[\alpha]_D$ in alc. ~69°
K. Freudenberg:	Pentaacetyl *l*-epicatechin	$C_{15}H_9O(OCOCH_3)_5$	m.p. 151~152°(4)		
The present author:	Pentaacetyl *tea* catechin	$C_{15}O_9O(OCOCH_3)_5$	m p. 151~152°		
K. Freudenberg:	Tetramethyl *l*-epicatechin	$C_{15}H_9O(OH)(OCH_3)_4$	m.p. 153~154°(4)		
The present author:	Tetramethyl *tea* catechin	$C_{15}H_9O(OH)(OCH_3)_4$	m.p. 153~154°		

As regards the chimecal constitution of catechin, the formula proposed by A.

G. Perkin and E. Yoshitake in 1902[2] seems to be the most reliable one and agrees best with various observations.



Catechin
(Perkin and Yoshitake)

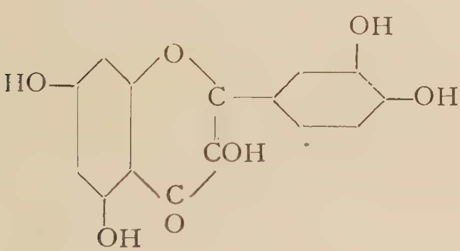


Quercetin

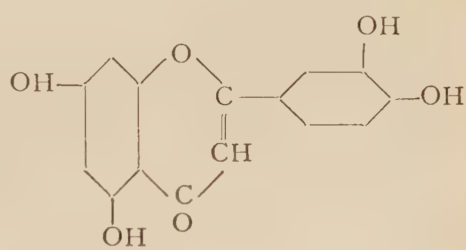
This assumption is chiefly based upon the close relationship existing between quercetin and catechin.

Leter, Freudenberg and his co-workers succeeded in transforming crynidin[5] and qurecetin[6] into catechin and gave further evidence in support of the Perkin's formula. They also studied the stereochemical relation of various kinds of catechins and classified[4] them into *d*-catechin, *l*-catechin, *d*, *l*-catechin, *d*-epicatechin, *l*-epicatechin and *d*, *l*-epicatechin. As there are two saymmetrical carbons in the Perkin's formula, the existence of such isomerides can easily be explained.

The author observed also that the absorption spectrum of tea catechin resembles to that of quercetin but not of luteolin. (See Y. Shibata and K. Kimotsuki's report.[7])



Quercetin
(Flavonol)



Luteolin
(Flavon)

As this difference is simply due to the presence of one OH group attaching to the pyrone nucleus in quercetin, so it can be assumed that, the arrangement of hydroxyl groups in tea catechin is the same as that in quercetin.

See Figs. of absorption spectra as follows :—

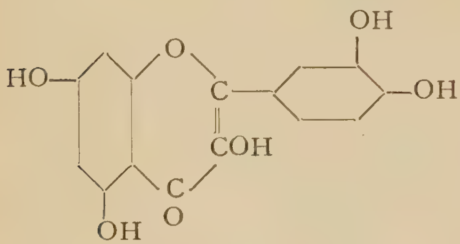
Fig. 3. Flavon (pentaacetyl quercetin)[7]

Fig. 4. Flavonol (querecetin)

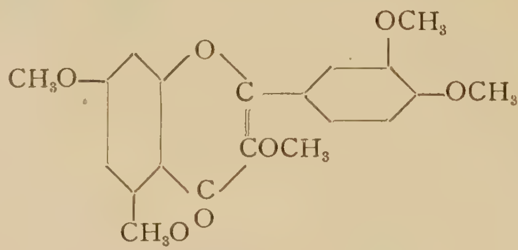
Fig. 5. Tea catechin

Further evidence in support of this view is given by the auther, viz. ;

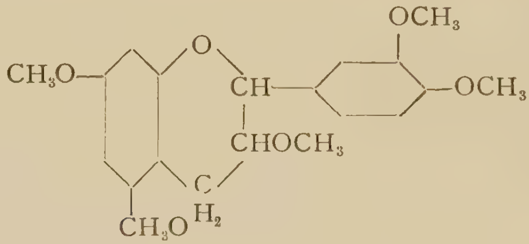
when pentamethyl-eqercetin is hydrogenated in glacial acetic acid, using platinum oxide as catalyser, according to the following scheme:



(1) Quercetin



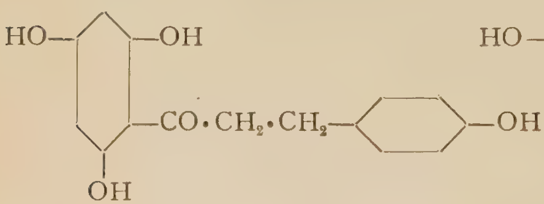
(2) Pentamethyl-quercetin



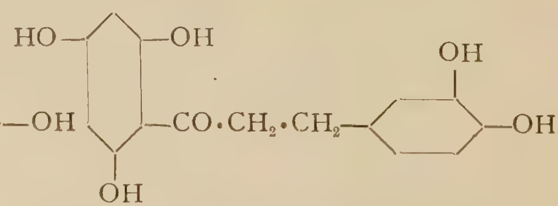
(3) Hydrogenated pentamethyl-quercetin

The hydrogenated product (3) shows exactly the same absorption spectrum (Fig. 6) with that of tea catechin and also with its methyl derivative. In this case, the methylation process seems to have no influence upon the absorption spectrum.

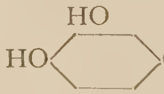
The question arises whether tea catechin might not be a series of phloretin, having the following formula:—



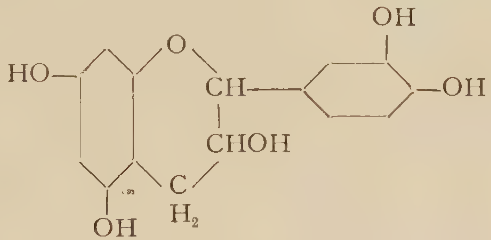
Phloretin ($C_{15}H_{14}O_5$)



Hypothetical formula for tea catechin ($C_{15}H_{14}O_6$)

However, such a hypothesis does not hold good because, (1) the absorption spectrum of phloretin is different from that of tea catechin, and further, (2) phloretin is decomposed into phloroglucinol and phloretic acid HO $CH_2 \cdot CH_2COOH$, when boiled with concentrated potash but in the case of tea catechin such a substance as  $CH_2 \cdot CH_2 \cdot COOH$ could not be detected.

The author believes thus, that the catechin formula proposed by Perkin and Freudenberg has found further support by the spectrographic studies of the auther, and tea catechin, as one of the stereoisomers, must be represented by the same formula.



Tea catechin

Experimental.

(1) The Isolation of Tea Catechin from Green Tea.

The material used in this experiment was the green tea of superior quality, prepared in Kyoto prefecture. It was finely powdered and repeatedly extracted with boiling ethyl acetate. The extract was evaporated and the residue was taken up in water. The aqueous solution thus obtained was now treated with neutral lead acetate, warmed for a short time on a water-bath and filtered quickly. The filtrate was then decomposed with hydrogen sulphide, again filtered and evaporated under a diminished pressure. The brown syrup obtained in this way was extracted with alcohol, the alcoholic solution was evaporated and the remaining syrup was dissolved in hot water, filtered, and the filtrate was concentrated under a diminished pressure to a small volume. After standing it for a short time, a white crystalline mass separated out, which was collected, washed with water and after drying on a porous tile it was Soxhlet apparatus with chloroform. The insoluble brown coloured crystalline mass, was then dissolved in hot water and filtered. From the filtrate, colourless glistening prismatic crystals of tea catechin separated out, which after recrystallization from hot water melted constantly at 237—238° (uncorr.) The yield was about 0.14% of the green tea used.

(2) Chief Properties of Tea Catechin.

The crystalline form of tea catechin obtained from aqueous solution is shown in Fig. 1, and that separated from alcoholic solution, in Fig. 2. The aqueous solution of tea catechin has an astringent taste. It gives a greenish blue colour with ferric chloride, which gradually changes to brownish yellow and finally forms a brown precipitate. When a few crystals of this substance are brought in contact with a pine shaving, moistened with concentrated hy-

drochloric acid, a reddish purple stain, characteristic of phloroglucinol is produced. It gives a reddish yellow colour with concentrated sulphuric acid, forming a precipitate. It is precipitated by lead acetate but not by gelatine. When bromine water is added to the aqueous solution a yellow precipitate is produced. When the aqueous solution is treated with lime water red colouration is first produced, forming a precipitate afterwards.

(3) Estimation of Rotatory Power.

The estimation was carried out with 95% alcoholic solution of tea catechin.

$$[\alpha]_D = \pm \frac{\alpha \times 100}{c \times l}$$

α	observed rotation,	$-0.69°$
c	concentration,	1%
l	length of tube in decimeters,	1

$$[\alpha]_D^{25} = -\frac{.69 \times 100}{1 \times 1} = -69°$$

(4) Elementary Analysis.

The crystals obtained from aqueous solution were dried in the air to constant weight and analysed as follows:

		CO_2	H_2O	C.	H.
Sample I.	3.412 mg.	7.745 mg.	1.568 mg.	61.91%	5.11%
〃 II.	2.565〃	5.841〃	1.218〃	62.12〃	5.28〃
Average				62.02〃	5.20〃
Calc. for $C_{15}H_{14}O_6$				62.06〃	4.83〃

The analytical result agreed with $C_{15}H_{14}O_6$ and showed that there was no water of crystallization.

(5) The Determination of the Molecular Weight.

The determination of the molecular weight was carried out by Rast's camphor method(8) with the following result:

$$m = \frac{c \times w \times 100}{dW}$$

m	mol. wt.	
c	const.	400
w	subst. mg.	11.3
d	depres. of m.p.	9°
W	solvent mg.	159.7

$$m = \frac{400 \times 11.3 \times 100}{9 \times 159.7} = 314$$

Calc. for $C_{15}H_{14}O_6$ 290

(6) Derivatives of Tea Catechin.

(a) Acetyl Derivative.

Tea catechin (0.5 g.) was dissolved in acetic acid anhydride, to which sodium acetate anhydride (1,5 g.) was added and the mixture was heated on a water-bath for five hours. The acetylation product was then poured into water, filtered by means of a suction and washed with. After drying on a porous tile, it was dissolved in hot alcohol and filtered. After standing, white needle crystals were obtained: m.p. 151~152°. The analysis gave the following result.

		CO_2	H_2O	C.	H.
Sample I.	4.574 mg.	10.053 mg.	2.152 mg.	59.94%	5.22%
〃 II.	3.550〃	7.849〃	1.480〃	60.23%	4.63%
Average				60.14〃	4.93〃
Calc. for	$C_{25}H_{24}O_{11}=C_{15}H_9O(OCOCH_3)_5$			60.0 〃	4.8 〃

(b) Methyl Derivative.

The methylation was carried out by K, Freudenberg's method,[9] viz.; the crystal (1 g.) was dissolved in methyl alcohol (6 c.c.) and methylated in a usual way with dimethyl sulphate (3 c.c.) and 50% KOH (3 c.c.). The reaction product was poured into water (100 c.c.), filtered by means of suction and recrystallized from methyl alcohol. It froms convex-lens-like crystals: m.p. 153~154°. The result of analysis was as below:

		CO_2	H_2O	C.	H.
Sample I.	2.954 mg.	7.125 mg.	1.706 mg.	65.78%	6.41%
〃 II.	2.844〃	6.826〃	1.938〃	65.46〃	6.40〃
Average				65.62〃	6.41〃
Calc. for	$C_{19}H_{22}O_6=C_{15}H_9O(OH)(OCH_3)_4$			65.89〃	6.35〃

The same methyl derivative was also obtained by methylating the acetylated product in the same manner as above.

$$C_{15}H_9O(OCOCH_3)_5 \longrightarrow C_{15}H_9O(OH)(OCH_3)_4$$

(7) Decomposition of Tea Catechin with Concentrated Potash.

Tea catechin was boiled with 50% KOH at 180° for 30 munutes. After cooling, it was diluted with water, neutralized with an acid and then made weakly alkaline by adding sufficient sodium bicarbonate when it was filtered and extracted with ether. The ether extract was evaporated and treated with water. From this aqueous solution a quantity of crystalline plates was obtained, which melted at 214°.

It gave the reactions of phloroglucinol; mixed with pure phloroglucinol no depression of melting point was observed, so it was proved to be phloroglucinol.

The residue of the ether extract was acidified with acetic acid, and extracted with ether. From this extract a small quantity of needle-shaped crystals was obtained which gave a greenich blue colour with ferric chloride. But, owing to shortage of the material it was not further investigated.

(8) Oxidation of Tetramethyl Tea Catechin with Potassium Permanganate.

The oxidation was carried out according to the method of Perkin[10]. For this purpose, tea catechin tetramethyl ether was mixed with water, heated on a water-bath and a strong solution of permanganate was added drop by drop. The operation lasted two hours when the excess of permanganate was decomposed with sodium sulphite and filtered. The filtrate was neutralized with sulphuric acid and extracted with ether. From this etherial solution veratric acid separated out as fine prismatic crystals: m.p. 176~177°.

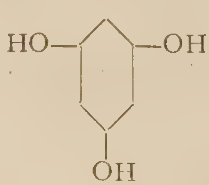
When pure veratric acid was mixed with the crystals obtained above, no depression of melting point was observed, hence it was proved to be veratric acid.

(9) Preparation of Veratric acid.

For this purpose, the alcoholic solution of vanillin (2 g.) was mixed with alcoholic potash (0.74 g.) and evaporated to dryness. The dried residue was now treated with methyl alcohol (4 c.c.) and methyl iodide (2.5 g.) and heated on a water-bath. After cooling, the reaction mixture was extracted with ether, and the etherial solution was evaporated off. The residue thus obtained was mixed with some water and a 5% permanganate solution was added drop by drop, heated on a water-bath and filtered. The filtrate was now concentrated to a small volume and acidified with dilute hydrochloric acid, when veratric acid separated out as prismatic crystals, which after recrystallization from dilute alcohol melted constantly at 178°.

(10) The Decomposition of Phloretin.

In order to compare tea catechin $C_{15}H_{14}O_6$ and phloretin $C_{15}H_{14}O_5$, phloretin was boild with 50% KOH in the same way as tea catechin was treated.

Phlorhglucinol 

and phloretic acid

$CH_2 \cdot CH_2COOH$ (m.p. 128°) were obtained.

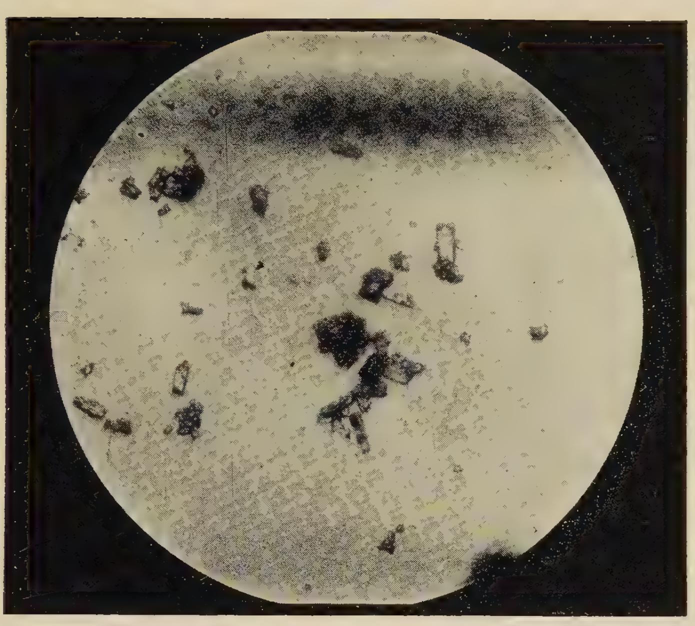


Fig. 1. Tea Catechin, from aq. sol.

1 : 100

Fig. 2. Tea Catechin, from alc. sol.

1 : 100

$^1/_{10000}$ mol.

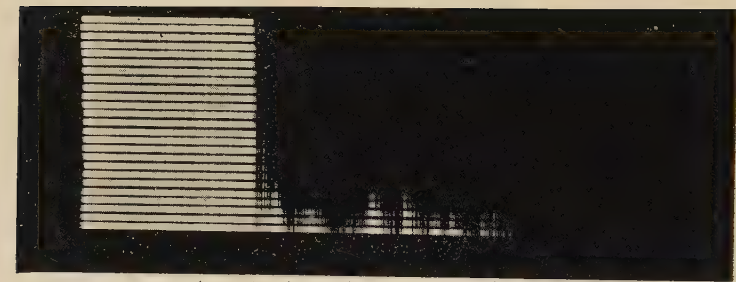


Fig. 3.

$^1/_{10000}$ mol.

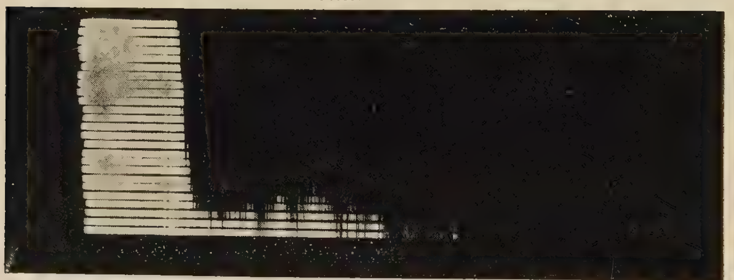


Fig. 4.

$^1/_{1000}$ mol.

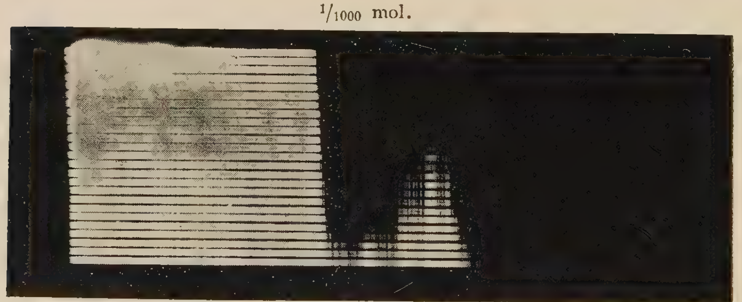


Fig. 5.

$^1/_{1000}$ mol.

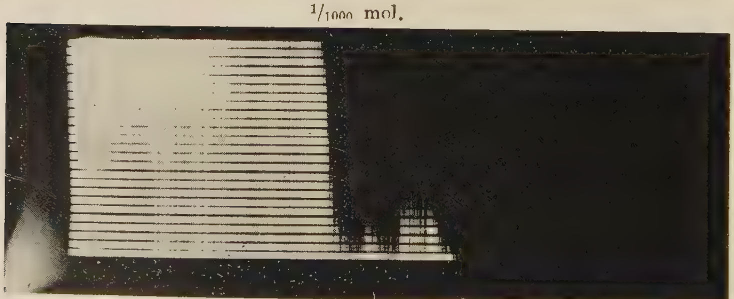
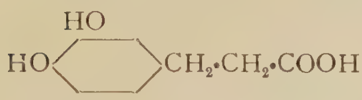


Fig. 5.

In the case of tea catechin, however, such a substance as



could not be obtained.

(11) Methylation of Quercetin.

Quercetin was methylated in the same way as tea catechin and penta-methyl-quercetin was obtained: m.p. 147～148°.[11]
Analytical result was as follows:

		CO_2	H_2O	C.	O.
Sample I.	3.236 mg.	7.678 mg.	1.575 mg.	64.71%	5.41%
〃 II.	2.416〃	5.699〃	1.166〃	64.33〃	5.36〃
Average				64.52〃	5.39〃
Calc. for	$C_{20}H_{20}O_7 = C_{15}H_5O_2(OCH_3)_5$			64.49〃	5.42〃

(12) Hydrogenation of Pentamethyl-Quercetin.

Pentamethyl-quercetin (0.5 g.) was dissolved in glacial acetic acid (5 c.c.) and hydrogenated in a usual way using platinum oxide[12] (0.1 g.) as catalyser, the operation lasted for 8 hours.

The absorpiion spectrum of the hydrogenated product is shown in Fig. 6.

The author expresses sincere thanks to Prof. U. Suzuki for his kind direction throughout this work and to Mr. S. Sakurai for his kindness in taking the photographs of absorption spectra.

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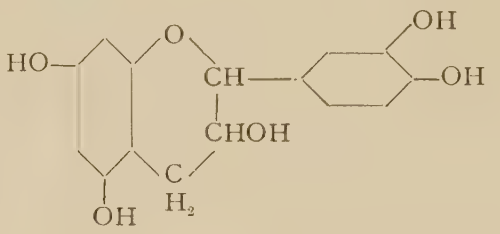
ON TEA TANNIN ISOLATED FROM GREEN TEA.

By

Michiyo Tsujimura.

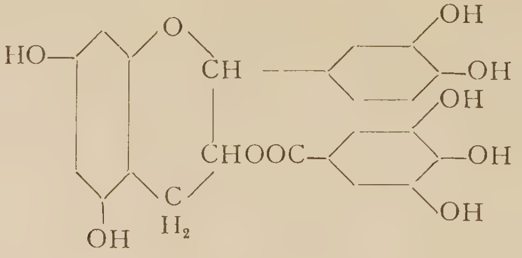
(Received September 8 th., 1930).

The author[1] has recently isolated Tea catechin from green tea and proposed the following formula as its constitution,



Tea catechin ($C_{14}H_{14}O_6$) m p. 237～238°, $[\alpha]_D^{25}$, = —69°.

Continuing the studies on tannin substance in green tea, the author has now succeeded in isolating a tannin, which is most probably the gallic acid ester of tea catechin, having the following formula:



Tea tannin ($C_{22}H_{18}O_{10}$).

The author gave it the name "Tea tannin". Tea tannin is an amorphous powder, easily soluble in water, and its aqueous solution has an acid reaction and an astringent taste. When pure, it is nearly colourless, but it is gradually oxidized in the air, to a reddish brown mass. On being boiled with 5% sulphuric acid, it gives gallic acid and a reddish brown substance which yields phloroglucinol, when heated with 50% potash, just in the same manner as in the case of tea catechin. Tea catechin could not be detected among the hydrolytic products of tea tannin, because the later is easily changed to a reddish brown substance by heating with sulphuric acid. The other properties

of tea tannin resemble to those of catechin and gallotannic acid. As tea tannin does not give the colour reaction with metallic magnesium and hydrochloric acid, no presence of flavon nucleus can be proved, The result of the analysis of tea tannin agrees with the formula $C_{22}H_{18}O_{10}$ and that of the acetyl derivative with $C_{36}H_{32}O_{17}$ or with $C_{22}H_{11}O_{10}(COCH_3)_7$. The molecular weight of the acetyl derivative was found to be 755 or 809, while the theory raquires 736.

The formation of tea tannin from tea catechin and gallic acid may be illustrated as follows ;

$$\underset{\text{Tea catechin}}{C_{15}H_{14}O_6} + \underset{\text{Gallic acid}}{C_7H_6O_5} - H_2O = \underset{\text{Tea tannin}}{C_{22}H_{18}O_{10}}$$

The specific rotation of tea tannin was found to be

$$[\alpha]_D^{23} = -162.5°,$$

and that of acetyl derivative,

$$[\alpha]_D^{23} = -100°.$$

The optical activity must be due to the presence of two asymmetrical carbon atoms in tea tannin, as is shown in the structural formula given above. The tea tannin isolated by the present author closely resembles to the tannin isolated by Deuss[(2)] in 1923. He gave the formulas $C_{20}H_{20}O_9$ and $C_{36}H_{36}O_{17}$ for his tannin and its acetyl derivative respectively. According to him this tannin has at least one CO-and eight OH-groups in its molecule, but no COOH-group. No detail was, however given by him whether phloroglucinol is found by the decompotion of his tannin, so it requires further investigation of his tannin, so it requires further investigation to undertsand the nature of the substance. Quite recently, Ryo Yamamoto and his co-workers[(3)] isolated tea catechin and a tannin from fresh tea leaves of Formosa. The latter substance is stated to have the formula $C_{17}H_{16}O_9$ and to give blue colour with ferric chloride, but apparently it is different from tea tannin.

Experimental.

(1) The Isolation of Tea Tannin from Green Tea.

The green tea was finely powdered and extracted at ordinary temperature with ethyl acetate containing 10% water. The extract was evaporated and the residue was taken up in water. When the aqueous solution was treated with a few drops of neutral lead acetate, a thick precipitate was formed, which was filtered off, and the clear filtrate was treated with so much neutral lead acetate as no more precipitate was formed. The yellowish white precipitate thus produced was collected by centrifugal process, washed with water, and decomposed with 10% sulphuric acid. The lead sulphate then was separated

by centrifugal machine and filtered, and the reddich yellow filtrate thus obtained was now repeatedly shaken with ethyl acetate. The united ethyl acetate solution was evaporated, the residue was dissolved in water and shaken with ether. The etherial solution, after being washed with water, was evaporated and the residue again dissolved in ether and after being decolorized with a little animal charcoal, evaporated on a water bath at low tamperature. The dried residue thus obtained was then extracted in a Soxhlet apparatus with chloroform ; the insoluble portion was dissolved in alcohol, filtered and evaporated. The residue was again dissolved in ether, decolorized with animal charcoal and washed with water. After repeating this operation, the etherial extract was evaporated, once more dissolved in ether and evaporated on a water-bath at low temperature,

(2) Important Properties of Tea Tannin.

The freshly prepared tea tannin is nearly colourless amorphous powder which gradually oxidizes in the air, to a reddish brown mass. It is soluble in water, alcohol, ether, ethyl acetate, aceton and glacial acetic acid, but insoluble in chloroform or benzene. The aqueous solution has an acid reaction and an astringent taste, and gives a blue coloration or blue precipitate with ferric chloride solution. It gives also the characteristic phloroglucinol reaction with pine shavings, moistened with concentrated hydrochloric acid. The aqueous solution gives, further, a yellow precipitate with bromine water, a white precipitate with gelatine, a reddish purple coloration with lime water, and a yellowish white precipitate with lead acetate.

For comparison, the chief reactions of tea tannin, catechin, gallic acid and gallotannic acid are shown in the following table :—

	Tea tannin	Catechin	Gallic acid	Gallotannic acid
Taste	astringent	astringent	——	astringent
$FeCl_3$	blue	green	blue	blue
Bromine water	yellow ppt.	yellow ppt.	no ppt.	no ppt.
Lime water	reddish purple	red	blue→red	grey
Phloroglucinol reaction	positive	positive	negative	negative
Gelathine	white ppt.	no ppt.	no ppt.	white ppt.
Heating with dil. acid	reddish brown ppt.	reddish brown ppt.	no ppt.	no ppt.

(3) Analysis of Tea Tannin.

	Sample	CO_2	H_2O	C %	H %
I.	3.246 mg.	6.595 mg.	1.583 mg.	55.57	5.42
II.	3.672	7.487	1.709	55.61	5.17
III.	3.255	6.601	1.543	55.31	5.23
	Average			55.49	5.29
	calc. for	$C_{22}H_{18}O_{10}+2H_2O$		55.23	4.60

(4) Specific Rotation of Tea Tannin.

$$[\alpha]_D = \pm \frac{\alpha \times 100}{c \times l}$$

α : observed rotation $= -3.25°$

c : concentration $= 2\%$ in 94% ethyl alcohol

l : length of tube in decimeter $= 1$

$$[\alpha]_D^{23} = -\frac{3.25 \times 100}{2 \times 1} = -162.5°$$

(5) Decomposition of Tea Tannin with 5% Sulphuric Acid.

[1] Gallic acid :— When tea tannin was boiled with 5% sulphuric acid for two hours, a reddish brown precipitate was formed. By sharking the latter with ether and evaporating the etherial extract, gallic acid was obtained in crystalline state, which gave blue colour with $FeCl_3$ and melted at 237°. Mixed with pure gallic acid, no depression of melting point was observed.

Analysis of Gallic Acid.

Sample	CO_2	H_2O	C%	H%
2.958 mg.	4.847 mg.	1.186 mg.	44.68	4.46
calc. for	$C_6H_2(OH)_3CO_2H+H_2O$		44.67	4.29

[2] Phloroglucinol :— After the reddish brown precipitate, obtained as above, was heated with 50% KOH at 180° for half an hour, it was cooled, diluted with water, neutralized with sulphuric acid and then extracted with ether. The etherial solution gave crystalline plates on evaporation, which gave all characteristic reactions of phloroglucinol. The purified colourless crystals melted at 213° and the mixture with pure phloroglucinol showed no depression of melting point.

[3] Glucose :— The mother liquor of the reddish brown precipitate was neutralized with barium carbonate, concentrated on a water bath, and thus tested for glucose : no phenylosazone was formed. Glucose was absent.

(6) Acetylation of Tea Tannin.

The acetylation was carried out in the following two ways.

[1] 0.5 Gram tea tannin was dissolved in 5 c.c. acetic acid anhydride, cooled and treated with one drop of concentrated sulphuric acid ; the reaction started immediately. After several hours, the reacting mixture was poured into water, and the precipitate formed thereby was collected, washed with water and dried on a porous tile. For purification it was dissolved it hot alcohol and filtered while hot. On cooling, a white amorphous substance

separated out, and it was purified from the methyl alcohol solution.

[2] 0.5 Gram tea tannin was dissolved in acetic acid anhydride; 1.5g. sodium acetate anhydride was added, and heated on a water-bath under reflex cooler for five hours; the reaction mixture was poured into water and the precipltate formed was treated as above.

Analysis of the Acetylated Tea Tannin.

	Sample (1)	CO_2	H_2O	C%	H%
I.	3.565 mg.	7.510 mg.	1.566 mg.	57.45	4.88
II.	3.264	6.853	1.382	57.26	4.70
	Average			57.36	4.79
	calc. for	$C_{22}H_{11}O_{10}(COCH_3)_7+H_2O$		57.29	4.51

	Sample (2)	CO_2	H_2O	C%	H%
I.	3.961 mg.	8.408 mg.	1.624 mg.	57.89	4.56
II.	3.183	6.712	1.302	57.51	4.55
	Average			57.7	4.56
	calc. for	$C_{22}H_{11}O_{10}(COCH_3)_7+\frac{1}{2}H_2O$		57.98	4.42

(7) Determination of Acetyl Value.

[1] The determination was carried out by Freudenberg's method.(4)

Sample		N/5 NaOH	CH_3CO%
I.	0.1200 g.	5.3 c.c.	37.98
II.	0.1063	4.7 〃	38.42

[2] The same determination was repeated with Kögl and Postwsky's method,(5) using alcoholic potash for saponification.

Sample		N/70 NaOH	CH_3CO%
I.	11.3 mg.	7.3 c.c.	39.49
II.	12.5 〃	8.1 〃	39.79
	calc. for	$C_{22}H_{11}O_{10}(CH_3CO)_7+\frac{1}{2}H_2O$	40.40

(8) Specific Rotation of the Acetyl Derivative.

$$[\alpha]_D = \pm\frac{\alpha\times 100}{c\times l}$$

$\alpha=-1^\circ$

$c=1\%$ in benzene solution

$l=1$ d.m.

$$[\alpha]_D^{23} = -\frac{1\times 100}{1\times 1}$$

(9) Molecular Weight.

The determination was carried out by Rust's camphor method.(5)

Tea catechin $^{1}/_{1000}$ mol. alc. sol.

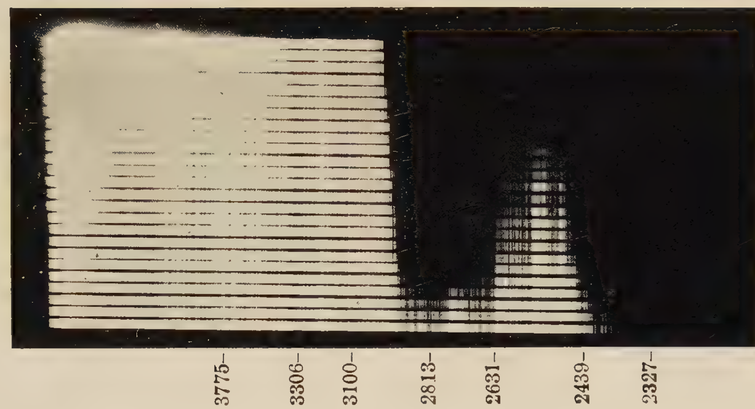


Fig. 1.

Tea tannin $^{1}/_{5000}$ mol. alc. sol.

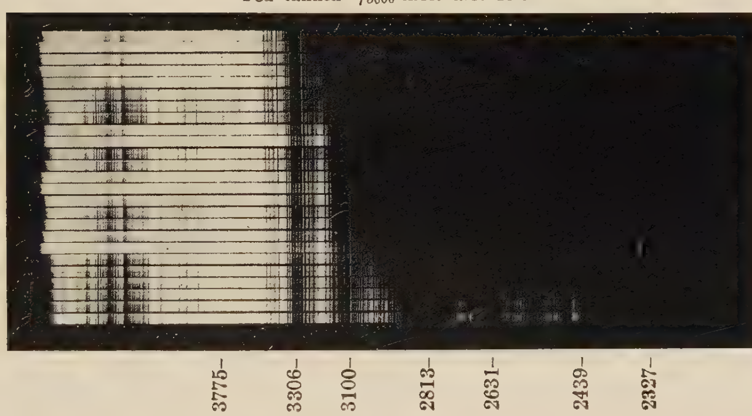


Fig. 2.

Gallic acid $^{1}/_{5000}$ mol. alc. sol.

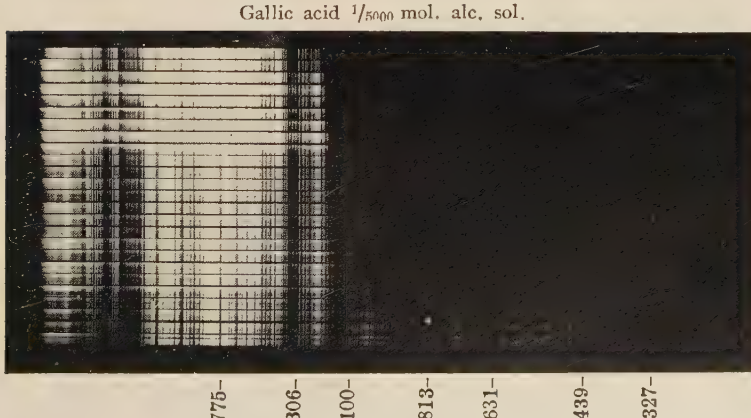


Fig. 3.

$$m=\frac{c\times w\times 100}{dW}$$

		I.	II.
m :	mol. wt.	I.	II.
c :	const.	400	
w :	subst. mg.	8.2	20.7
d :	depress. of m.p.	5°	4°
W :	solvent mg.	81	273.3

$$\text{I.}\quad m=\frac{400\times 8.2\times 100}{5\times 81.0}=809$$

$$\text{II.}\quad m=\frac{400\times 20.7\times 100}{4\times 273.3}=755$$

calc. for $C_{22}H_{11}O_{10}(COCH_3)_7$ 736

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